



U.S. Department of Energy Office of Science

Environmental Remediation Sciences Program

Field Investigations of Lactate-Stimulated Bioreduction of Cr(VI) to Cr(III) at Hanford 100H



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Koenigsberg*

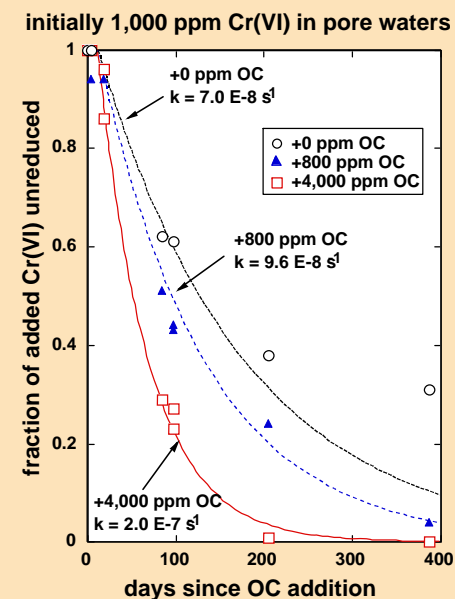
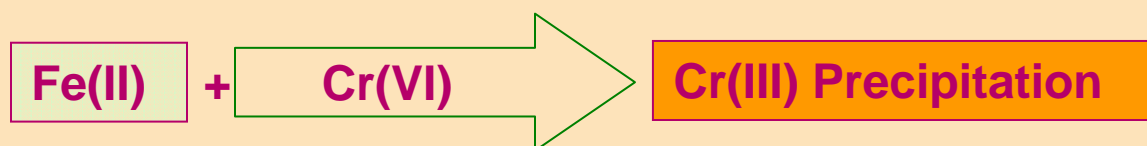
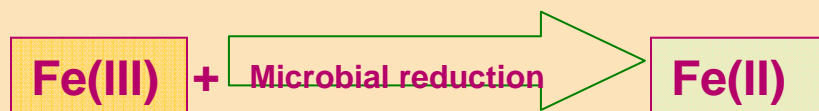
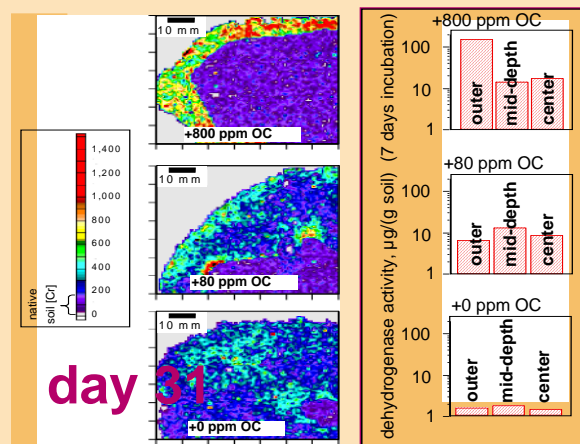
Mesoscale Studies on Cr(VI) Bioreduction that led to Field Studies

Jiamin Wan, Tetsu Tokunaga, Mary Firestone and Terry Hazen (NABIR supported 1998-2004)

Tokunaga, T. K. J. Wan, M. K. Firestone, T. C. Hazen, K. R. Olson, D. J. Herman, S. R. Sutton, and A. Lanzirotti. 2003. *In-situ* reduction of Cr(VI) in heavily contaminated soils through organic carbon amendment. J. Environ. Qual. 32:1641-1649.

Tokunaga, T. K., J. Wan, T. C. Hazen, E. Schwartz, M. K. Firestone, S. R. Sutton, M. Newville, K. R. Olson, A. Lanzirotti, and W. Rao. 2003. Distribution of chromium contamination and microbial activity in soil aggregates. J. Environ. Qual. 32:541-549.

Tokunaga, T. K., J. Wan, M. K. Firestone, T. C. Hazen, E. Schwartz, S. R. Sutton, and M. Newville. 2001. Chromium diffusion and reduction in soil aggregates. Environmental Science & Technology 35:3169-3174.



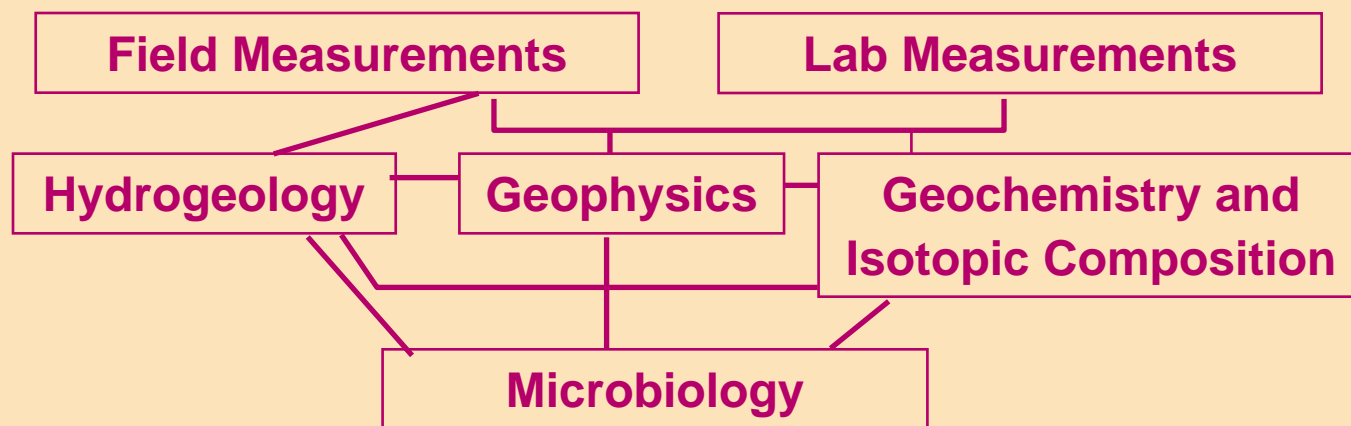
Multidisciplinary Team

Scientific Field	LBNL	PNNL	Regenesis
Microbiology	Terry Hazen, Eoin Brodie, Sharon Borglin, Dominique Joyner, Mary		
Hydrogeology	Firestone Boris Faybishenko, Jiamin Wan, Tetsu Tokunaga	Philip E. Long, Bruce Bjornstad	
Geophysics	Susan Hubbard, Ken Williams, John Peterson,		
Geochemistry	Mark Conrad	Tom Resch, Kirk Cantrell	
Field and technical support	Victor Gruol, Phil Rizzo	Darrell Newcomer	Steve Koenigsberg, Anna Willet, Kevin Lapus

Overall Objective

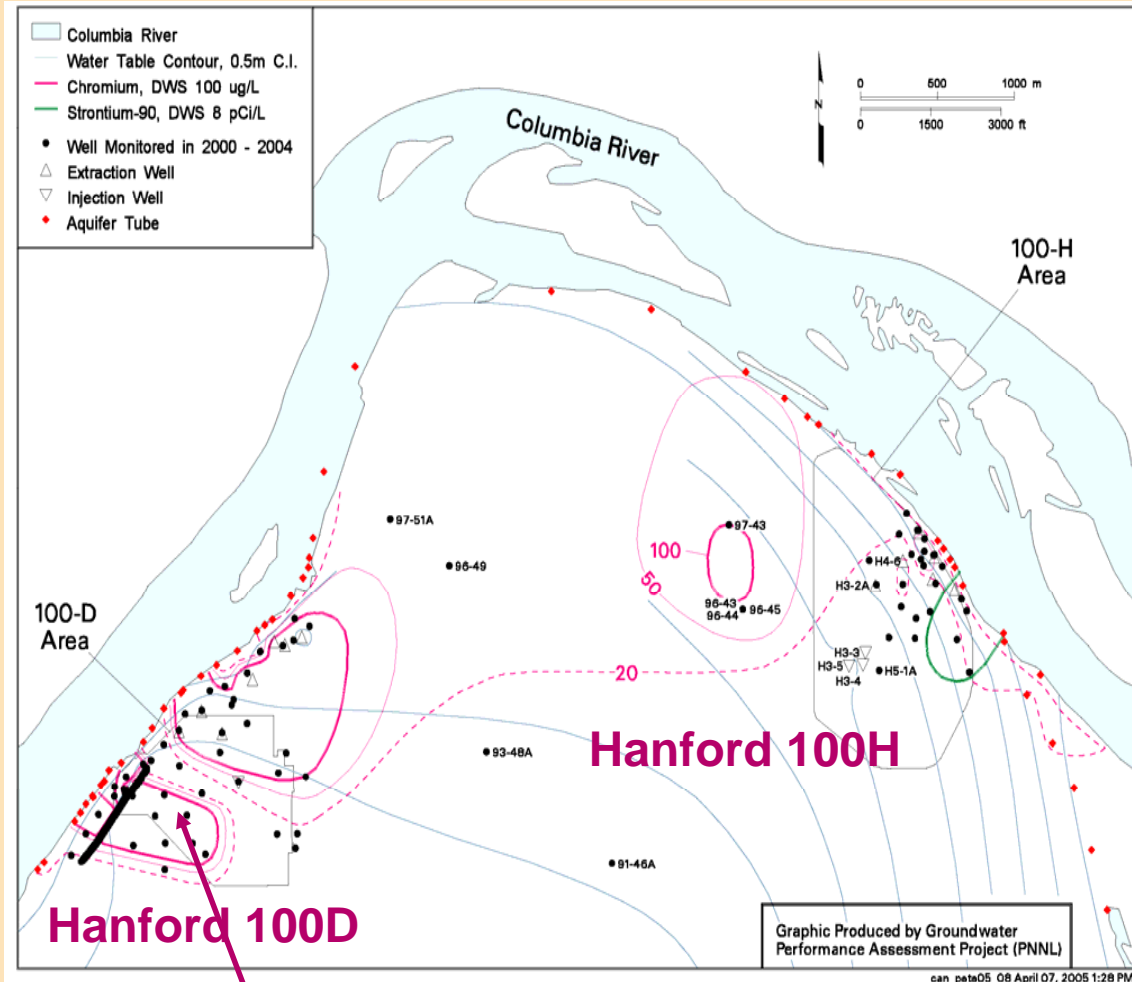
To carry out field investigations to assess the potential for immobilizing Cr(VI) in groundwater using lactate-stimulated bioreduction of Cr(VI) to Cr(III) at the Hanford 100H site, and to determine critical community structure changes and stressors that would enable control and predictions of fundamental biogeochemistry that enables this bioremediation strategy for Cr(VI)

Integrated Approach



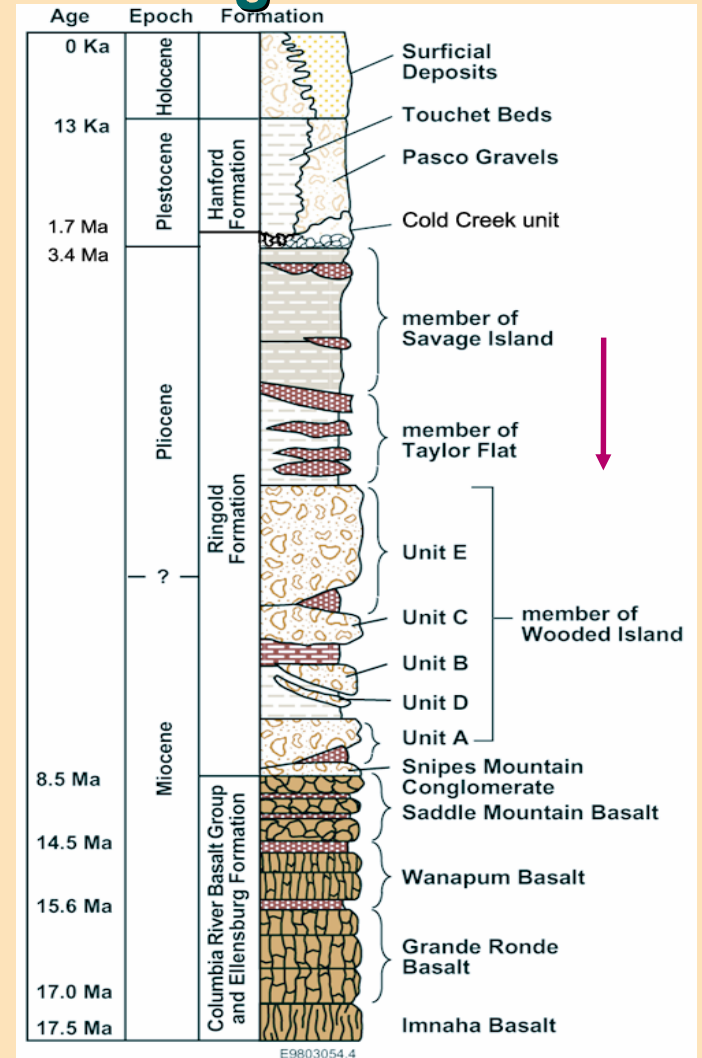
Hanford 100H Site Characterization

Cr Concentration Map



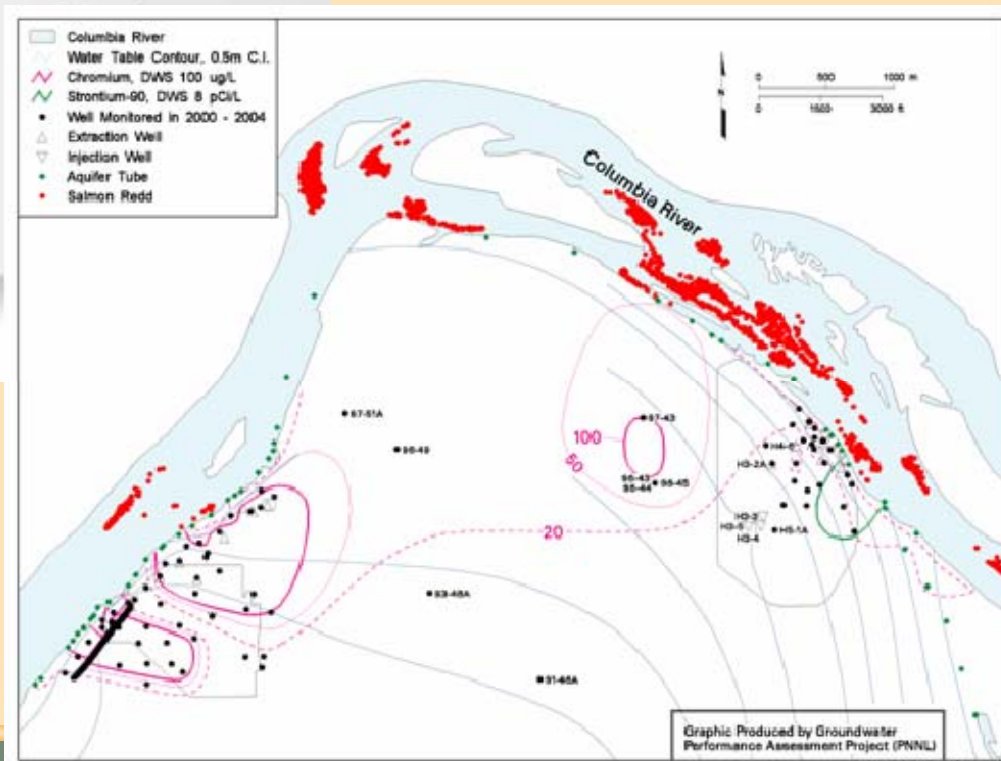
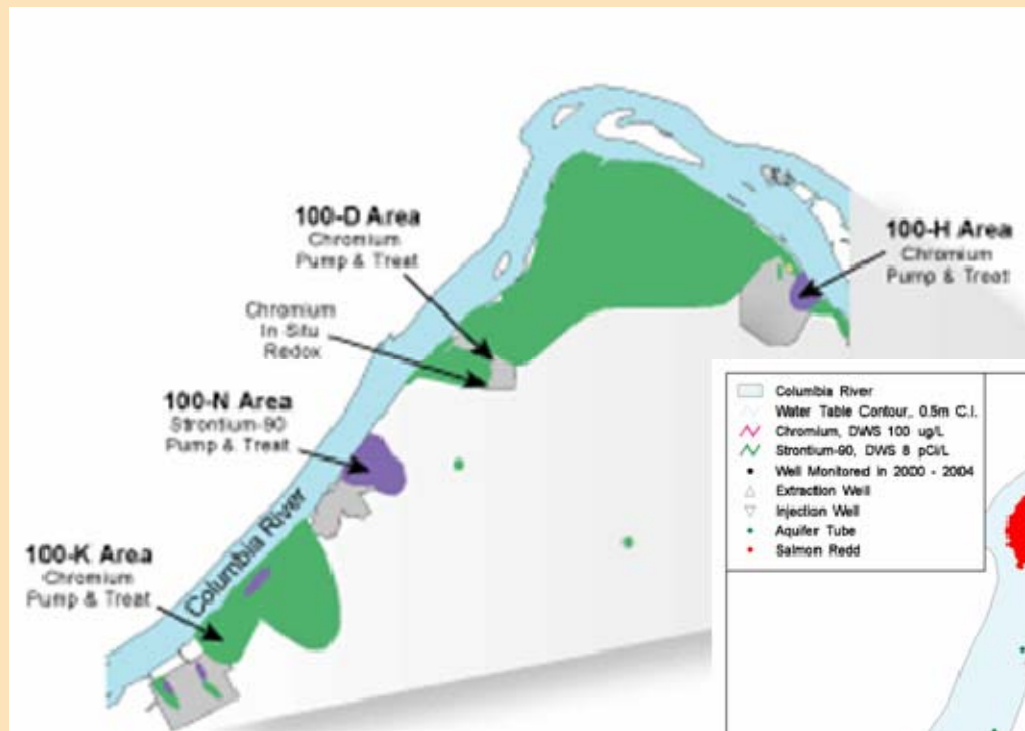
The Cr source is believed to be sodium dichromate (Na₂Cr₂O₇·2H₂O)

Lithological Column



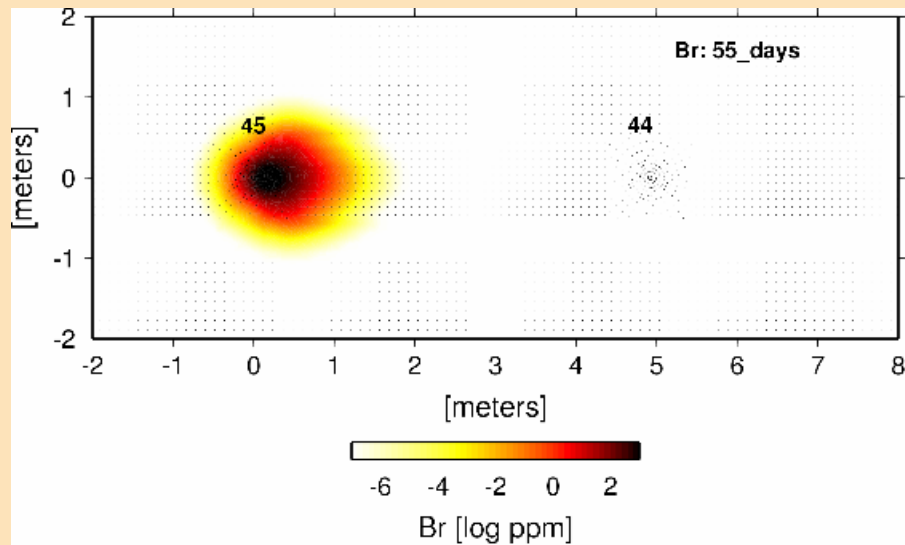
<http://esd.lbl.gov/ERT/hanford100h/>

100 Area Hexavalent Chromium Plumes

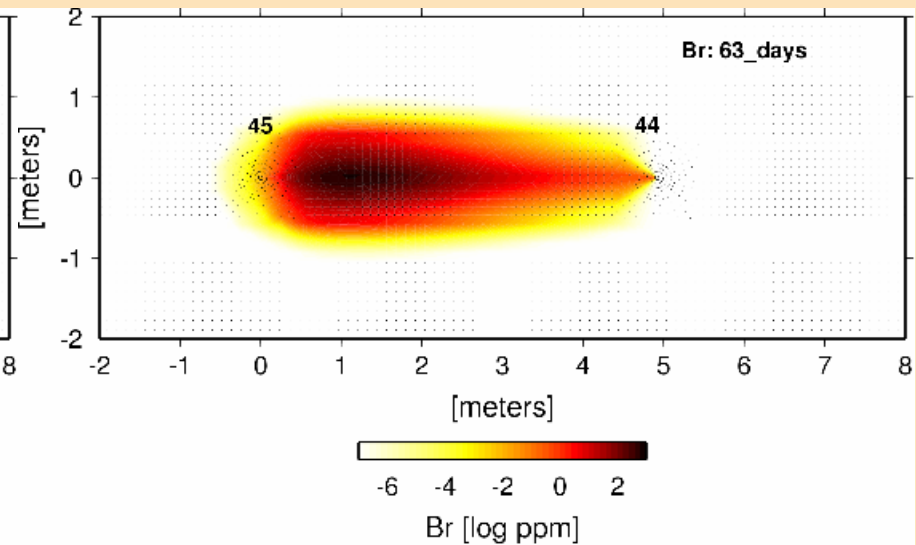


LiBr Injection (2/27/2004)

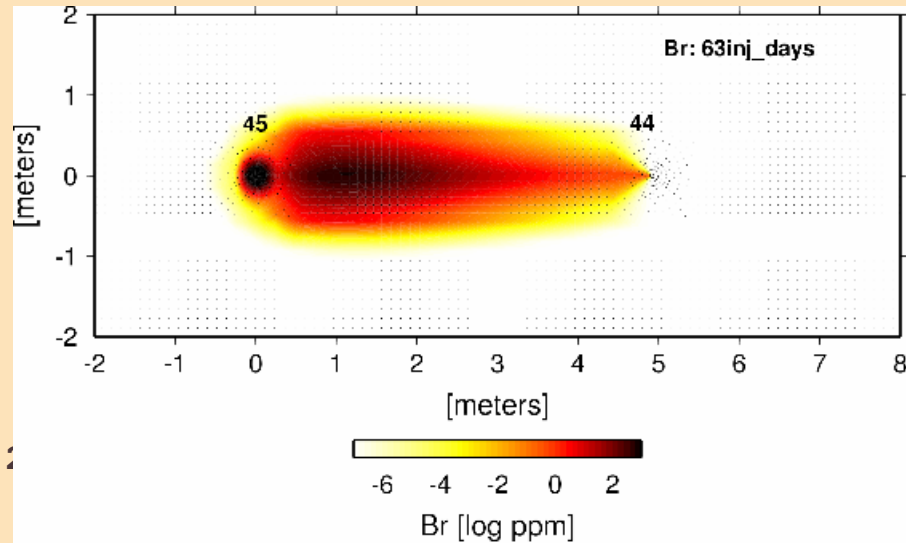
55 Days No pumping



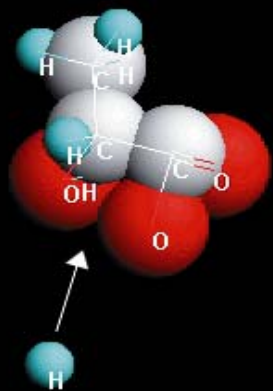
63 Days Pumping



63 Days Pumping +
2nd Injection



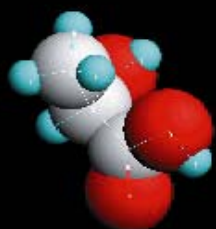
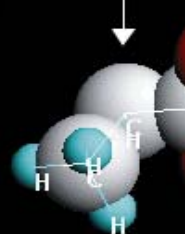
- LiBr Injection: 15.3 g/liter, 12 liters over 2 hours



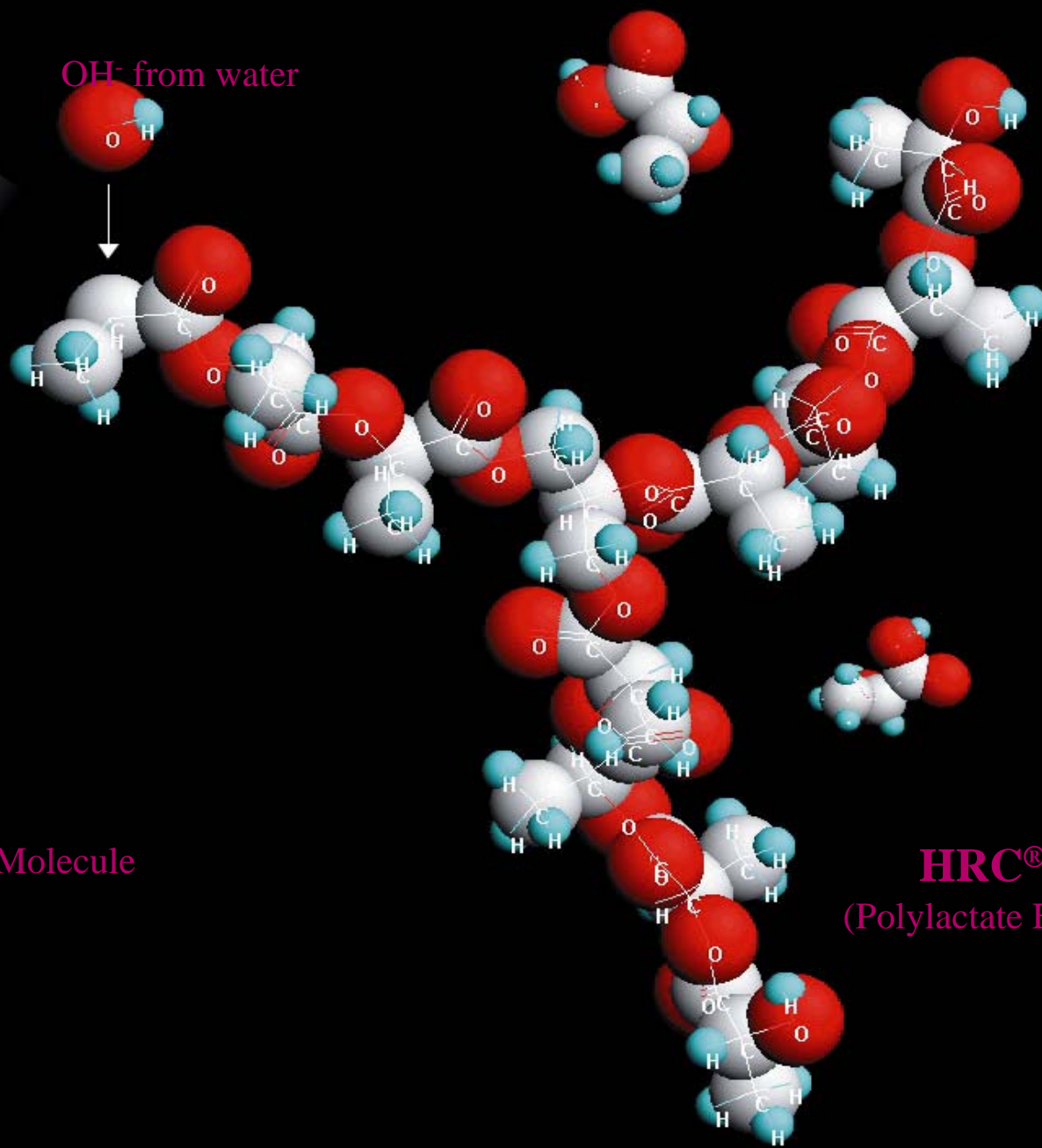
H^+ from water



OH^- from water

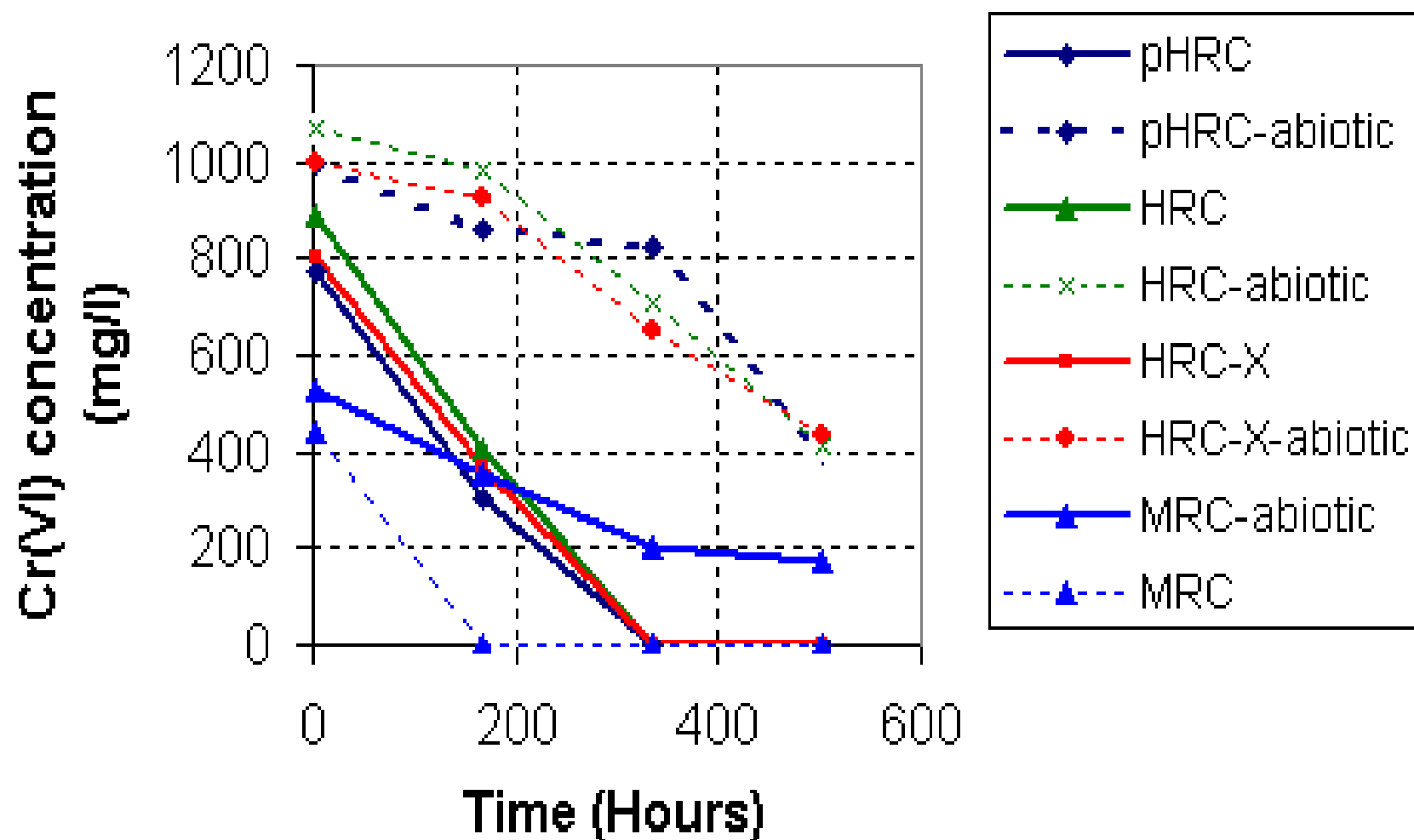


Lactic Acid Molecule

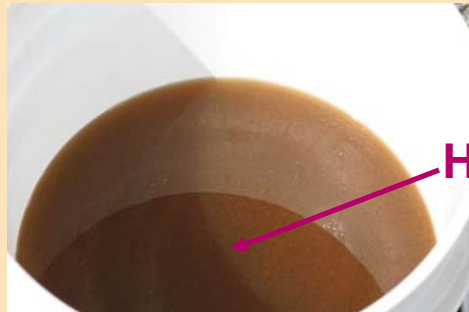


HRC[®]
(Polylactate Ester)

Lactate-Induced Bioreduction of Cr(IV)



Field HRC Injection Test



Injection of 40 lbs of ^{13}C -labeled HRC
Well 699-96-45, August 3, 2004

Pumping - 27 days
Well 699-96-44

**Injection at depths
of 44 ft to 50 ft**

**Hanford sandy gravel
and gravelly sand**

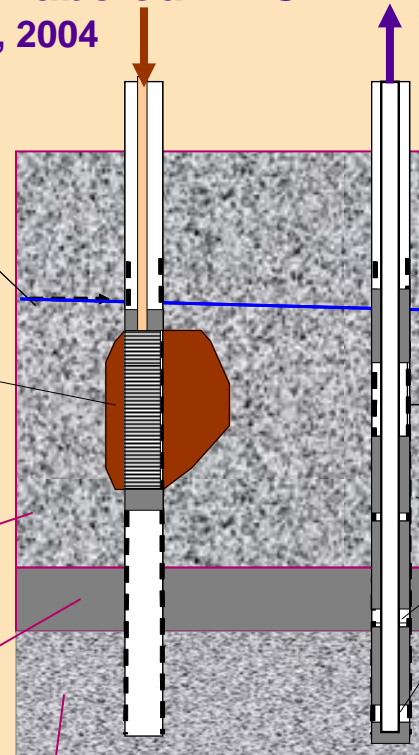
Ringold clay

Ringold silt

Groundwater level

Pumping

**Water
samplers**



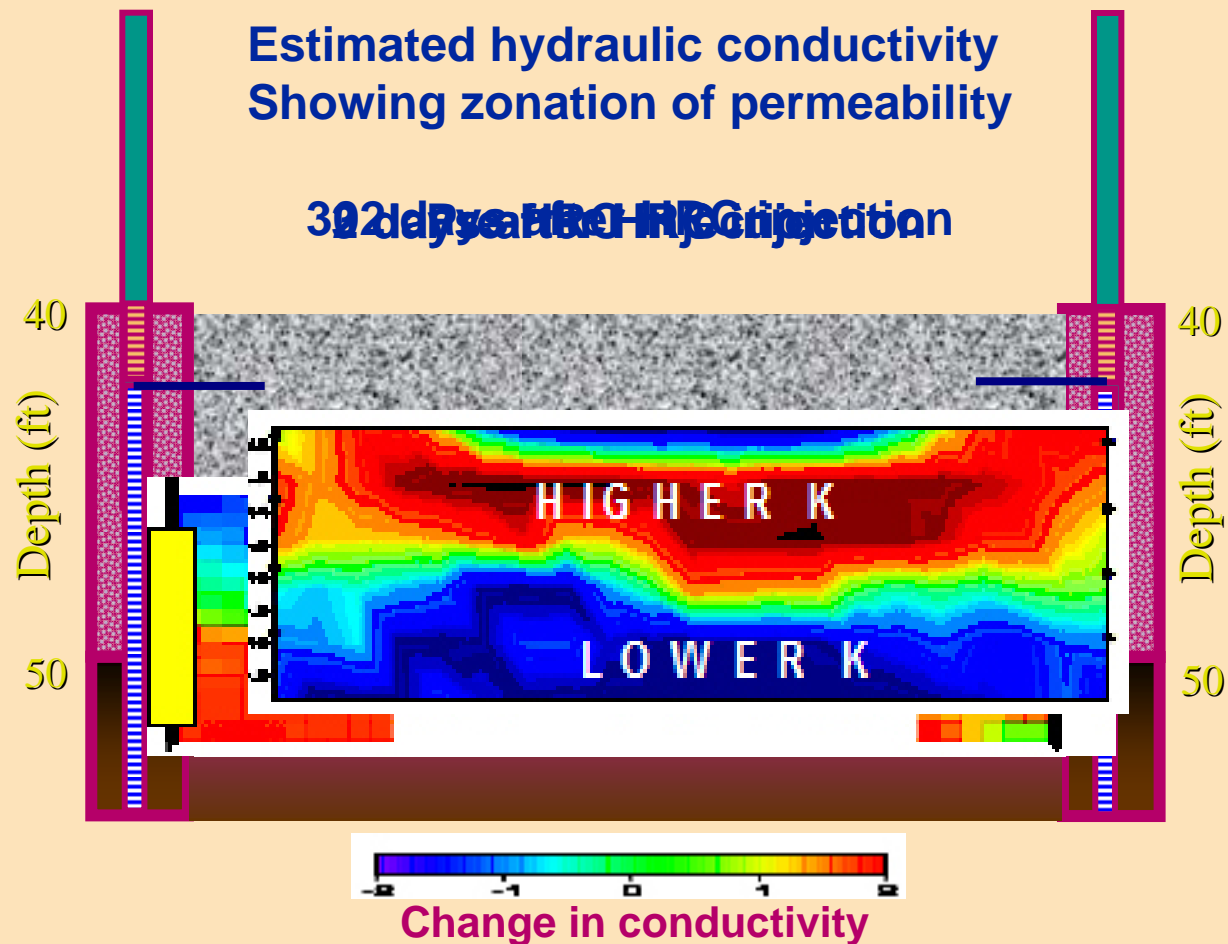
Non-invasive geophysical monitoring

Injection well

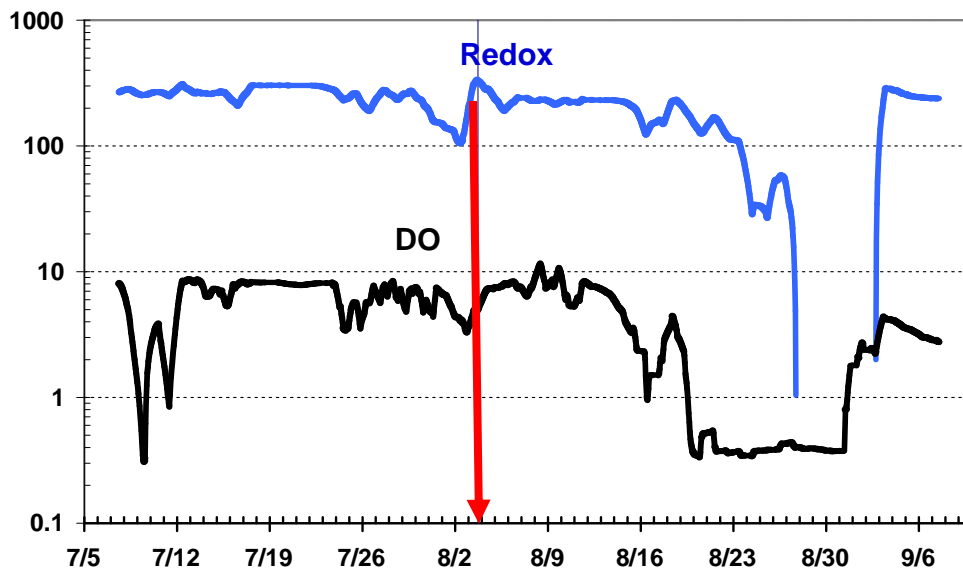
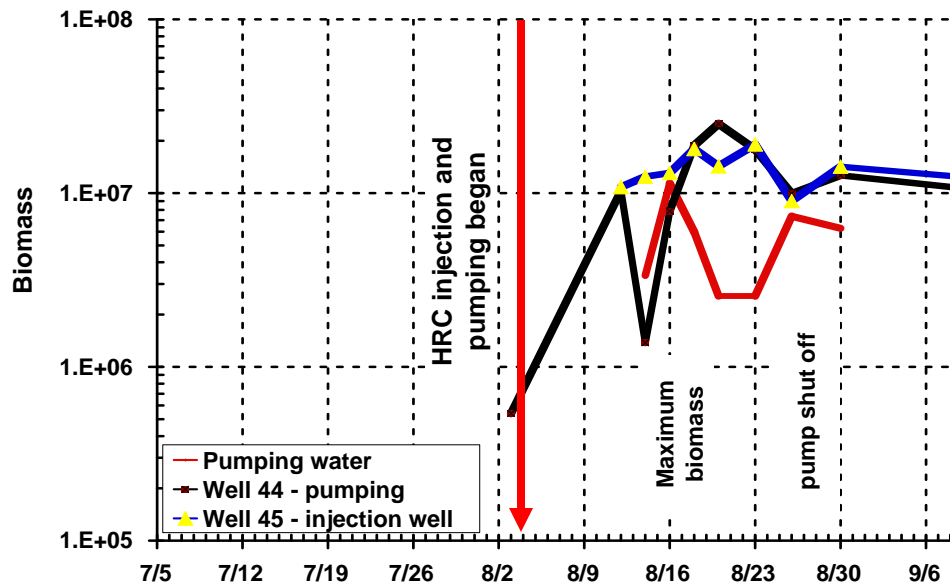
Monitoring well

Estimated hydraulic conductivity
Showing zonation of permeability

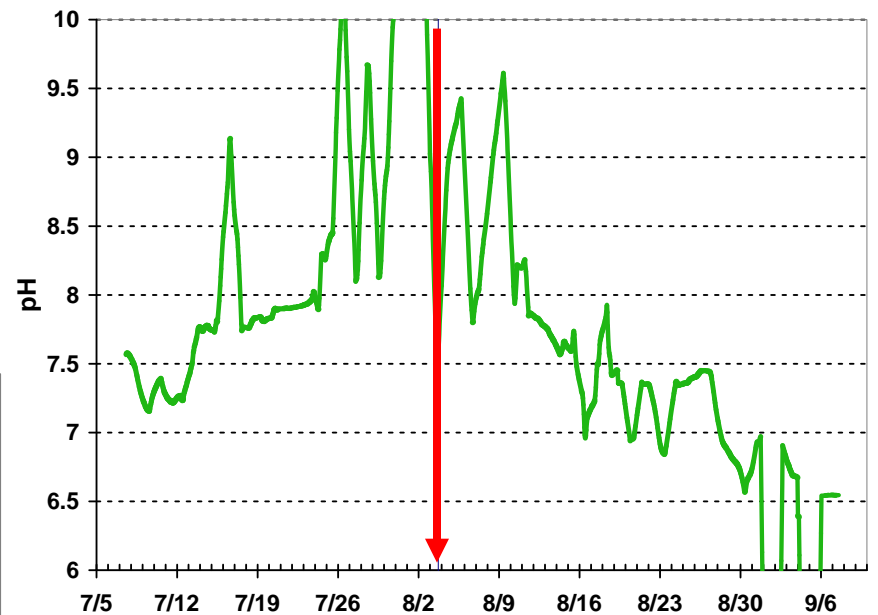
302 days after H₂O-NaCl injection



Results of HRC Biostimulation



D. vulgaris (direct fluorescent antibody)

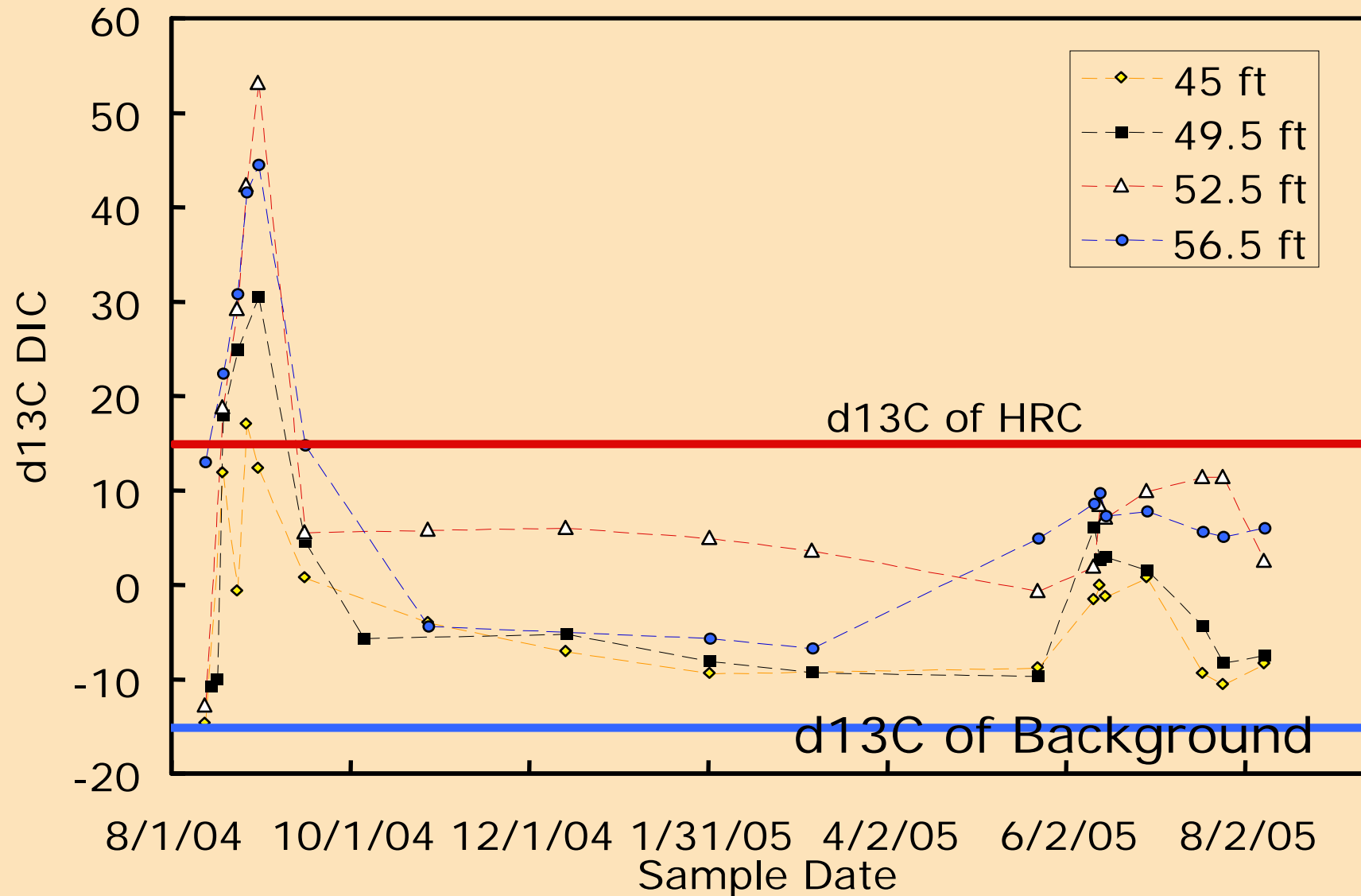


Redox dropped from 240 to -130 mV

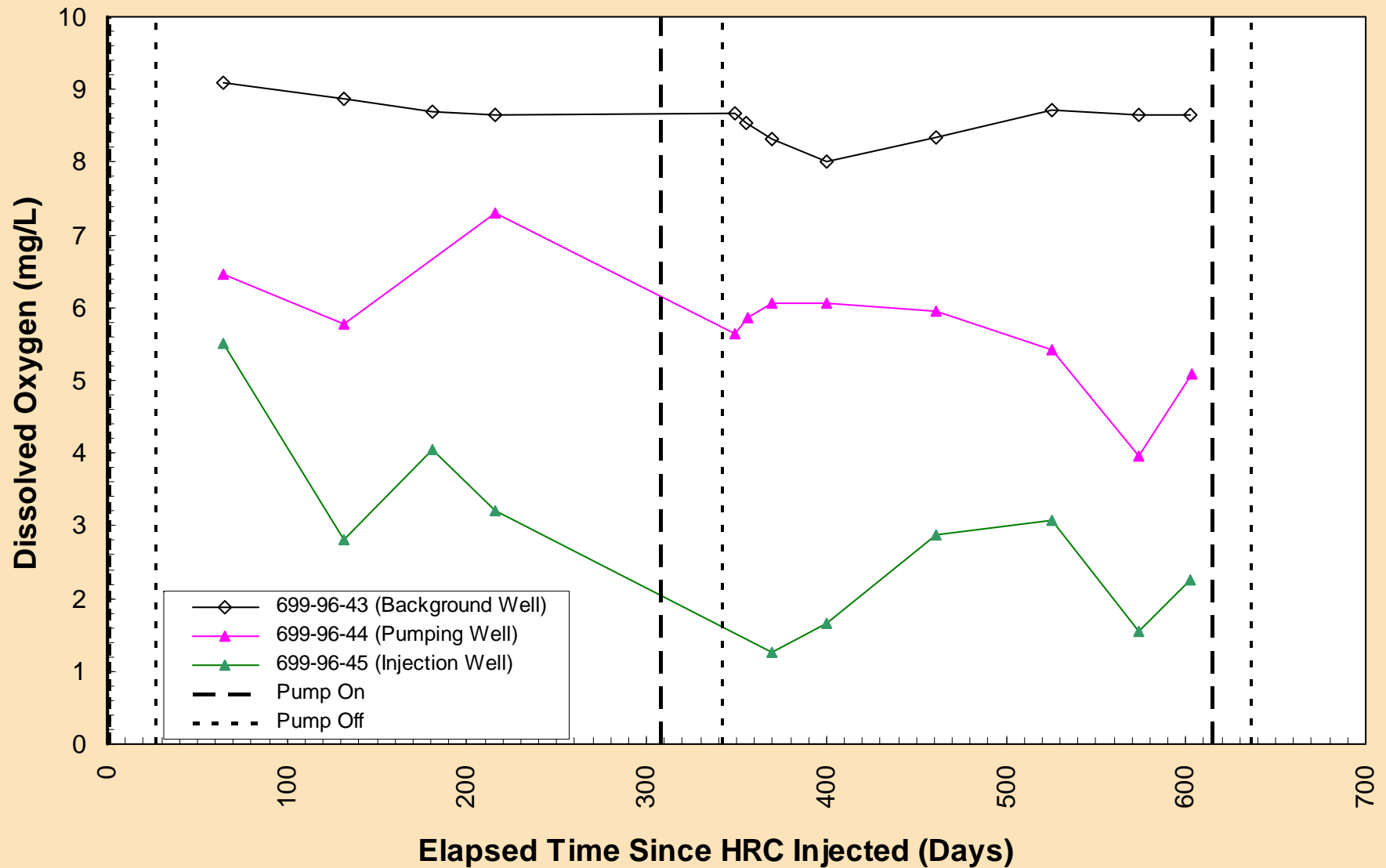
DO dropped from 9 mg/l (~100%) to 0.35 mg/l (4.5%)

Biogeochemical Evidence of Microbial Metabolism in Groundwater

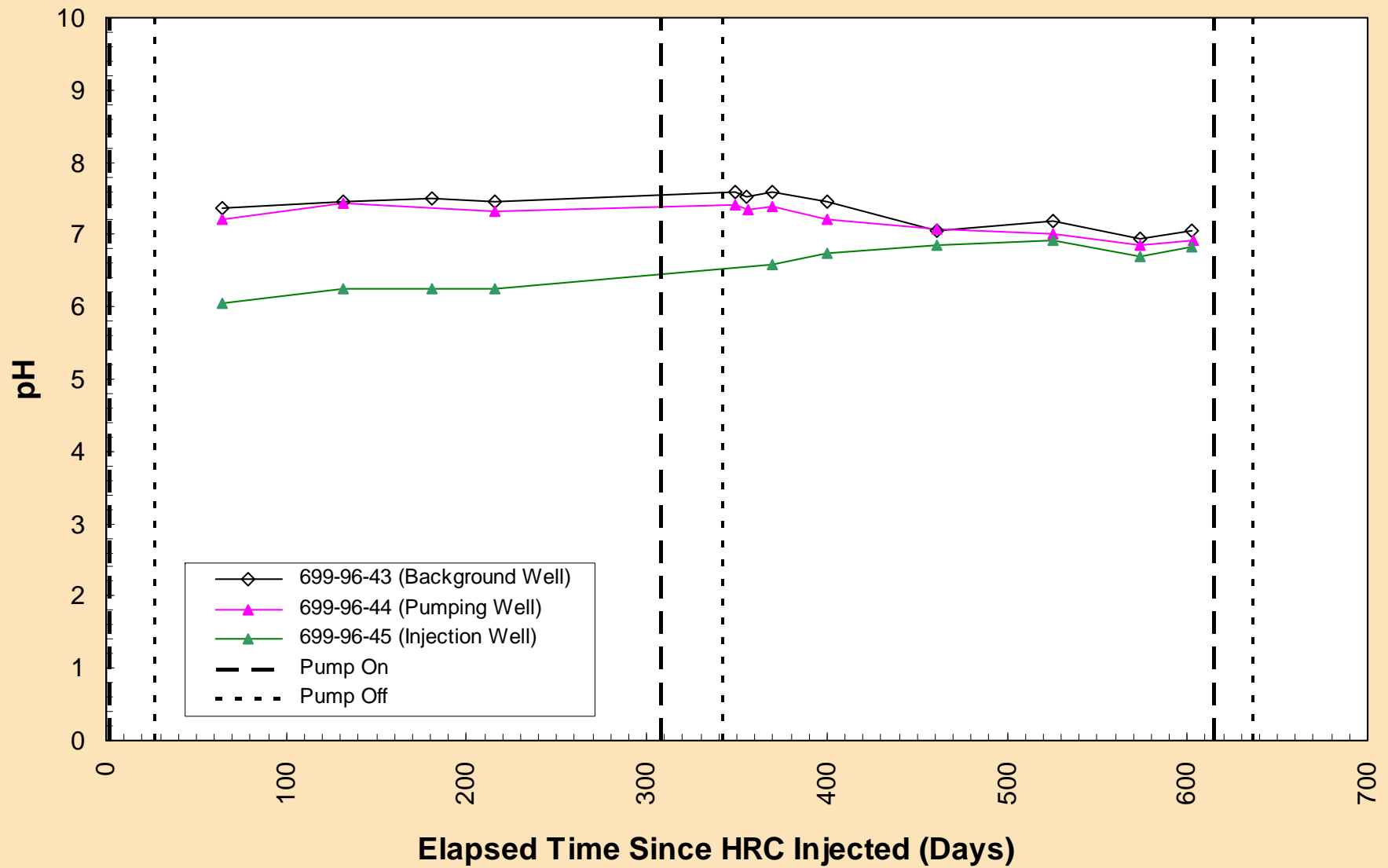
$\delta^{13}\text{C}$ of Dissolved Inorganic Carbon is Byproduct of HRC Metabolism



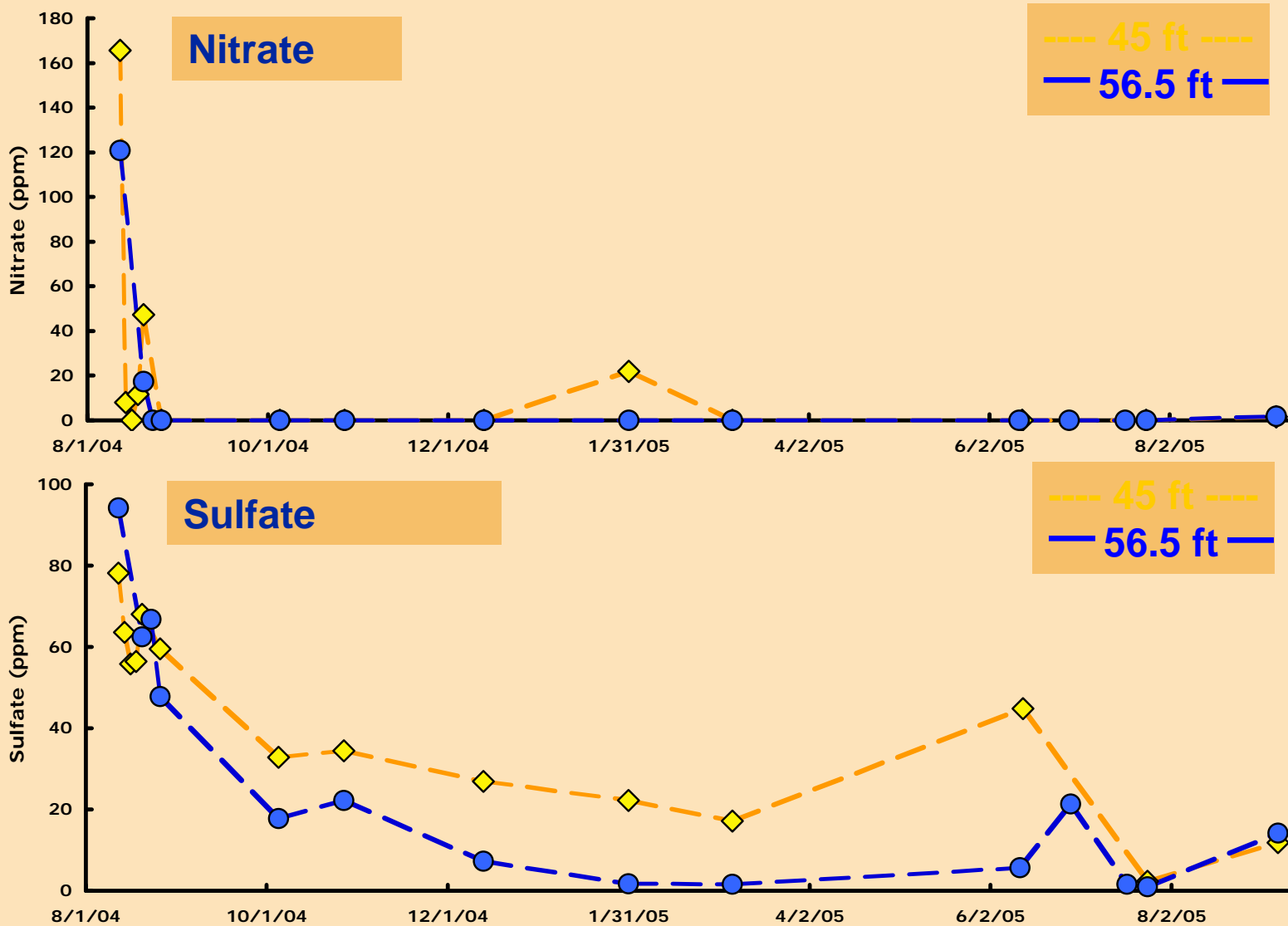
HRC Experiment, Monthly Sampling



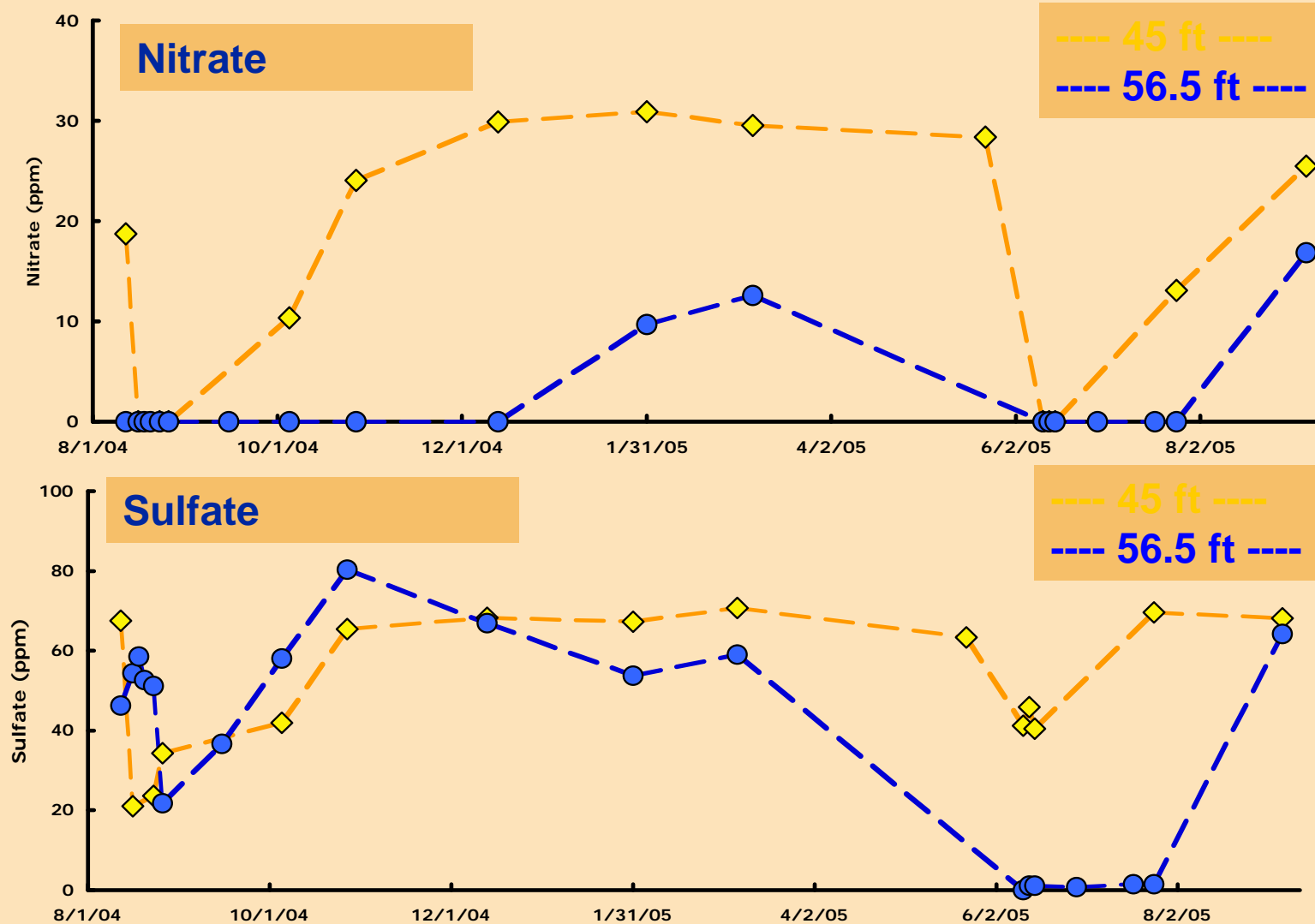
HRC Experiment, Monthly Sampling



Geochemistry - Injection well

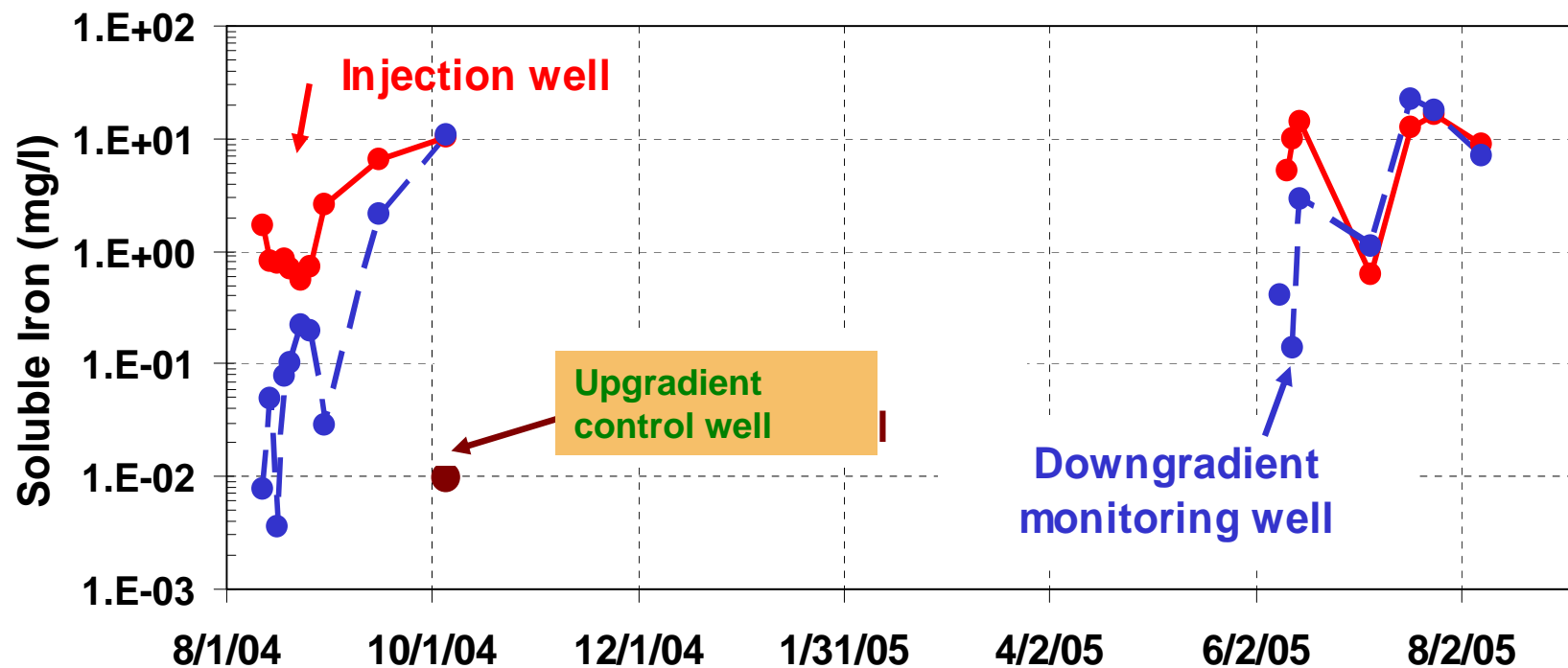


Geochemistry - Monitoring well

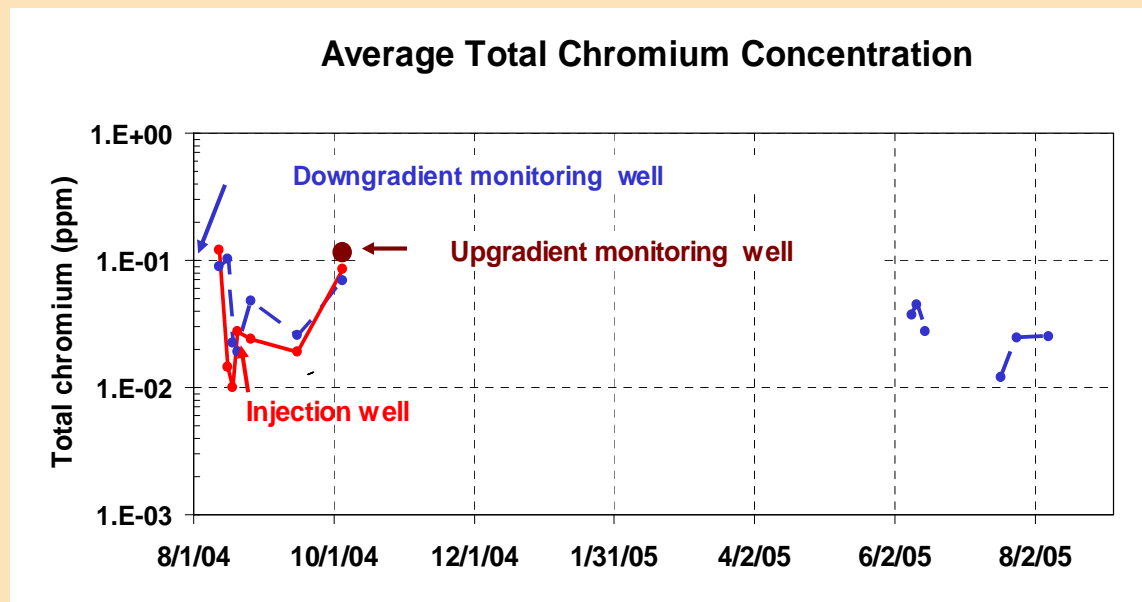
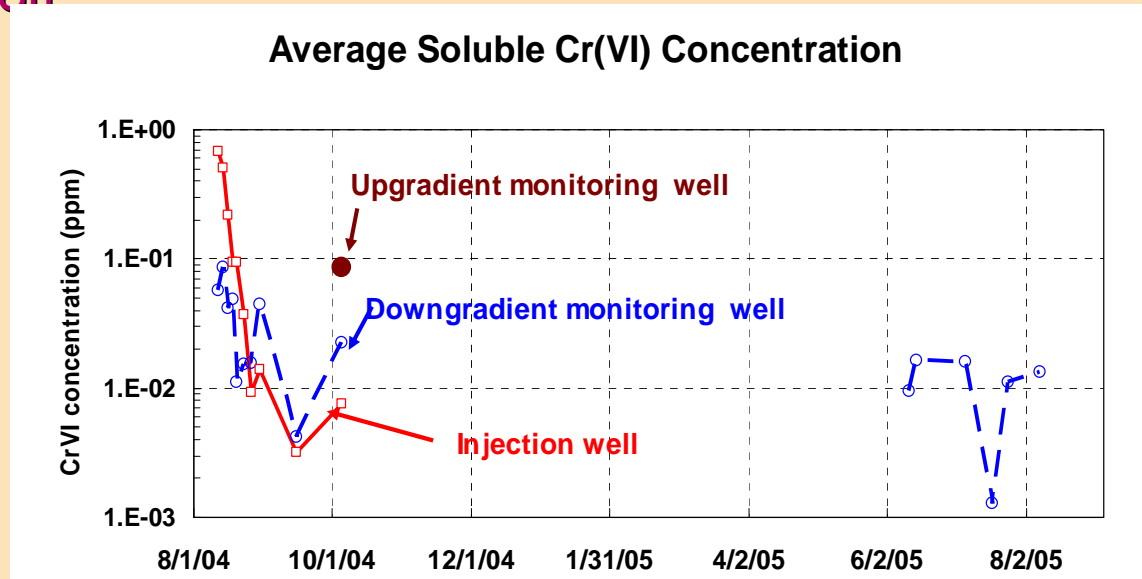


Geochemistry - Iron reduction

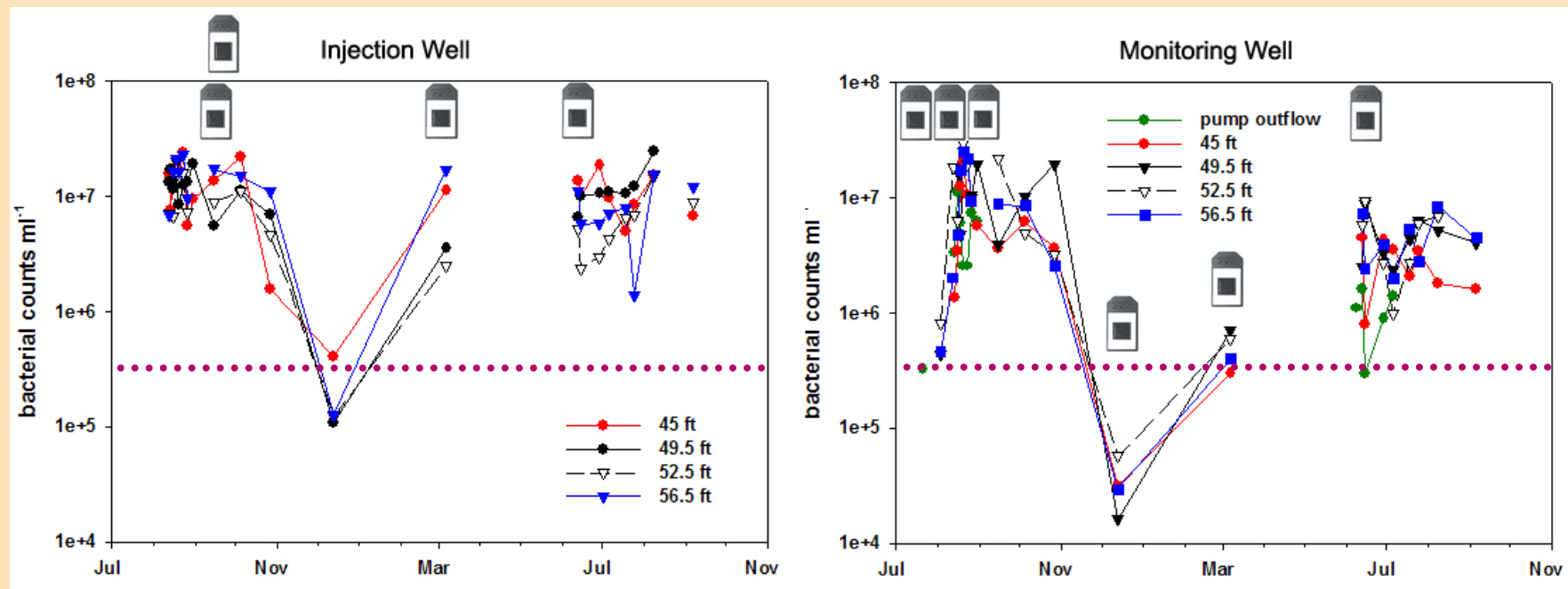
Average Soluble Iron, Fe(II), Concentration



Changes of Cr(VI) Concentration in Groundwater after HRC Injection



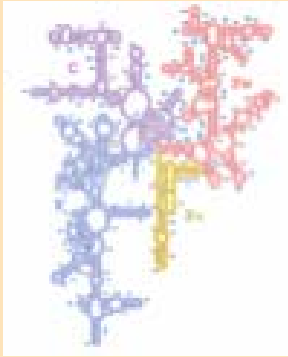
Bacterial biomass - Direct counts



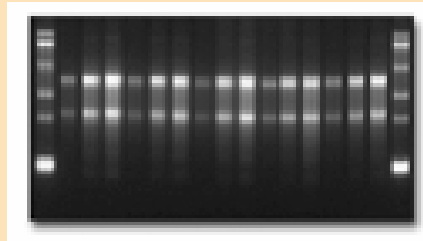
Bacterial biomass enriched rapidly by 2 orders of magnitude – remained elevated over one year later

PhyloChip

- PhyloChip – 500,000 probes (300k target 16S)



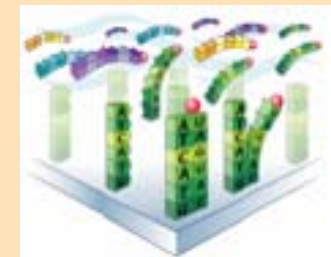
16S rRNA gene used as biomarker due to large database and availability of “universal” primers.



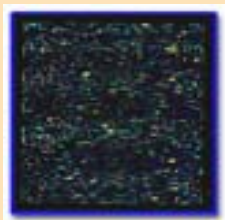
16S rRNA gene is amplified from genomic extract or 16S rRNA molecules are used directly



Amplicon pool fragmented, biotin labeled



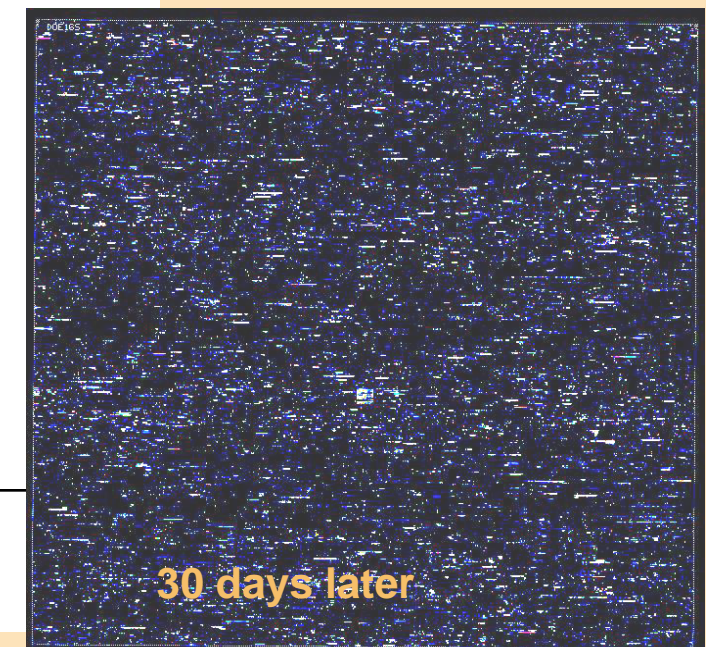
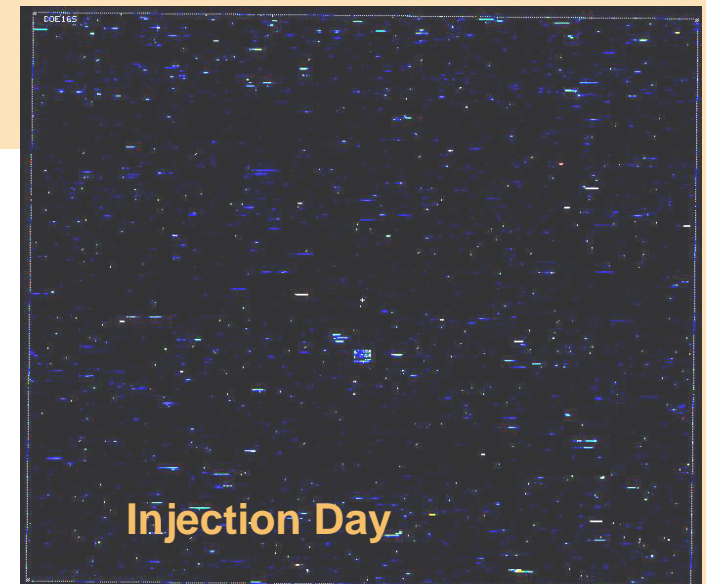
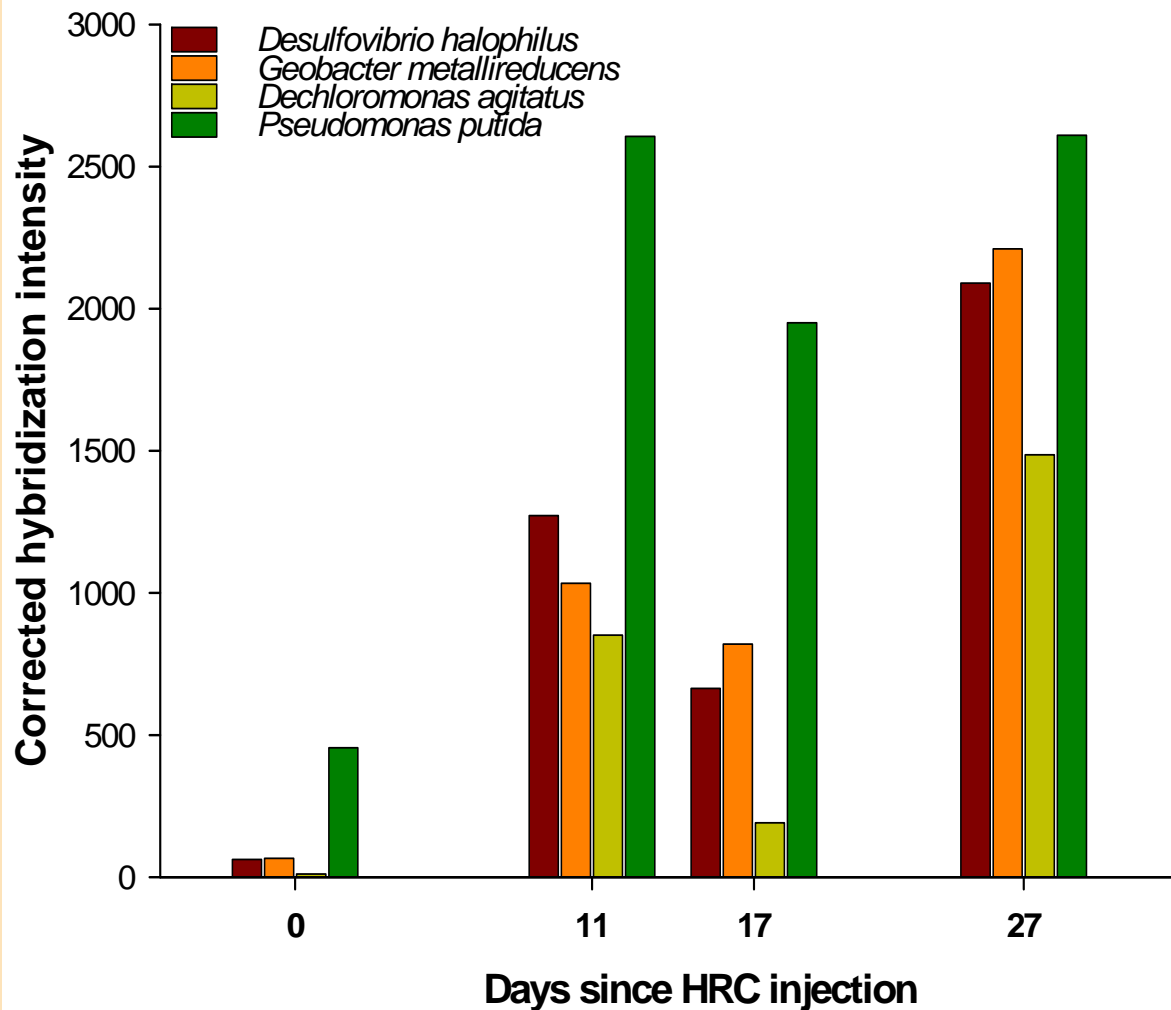
PhyloChip stained and washed using automatic fluidics station

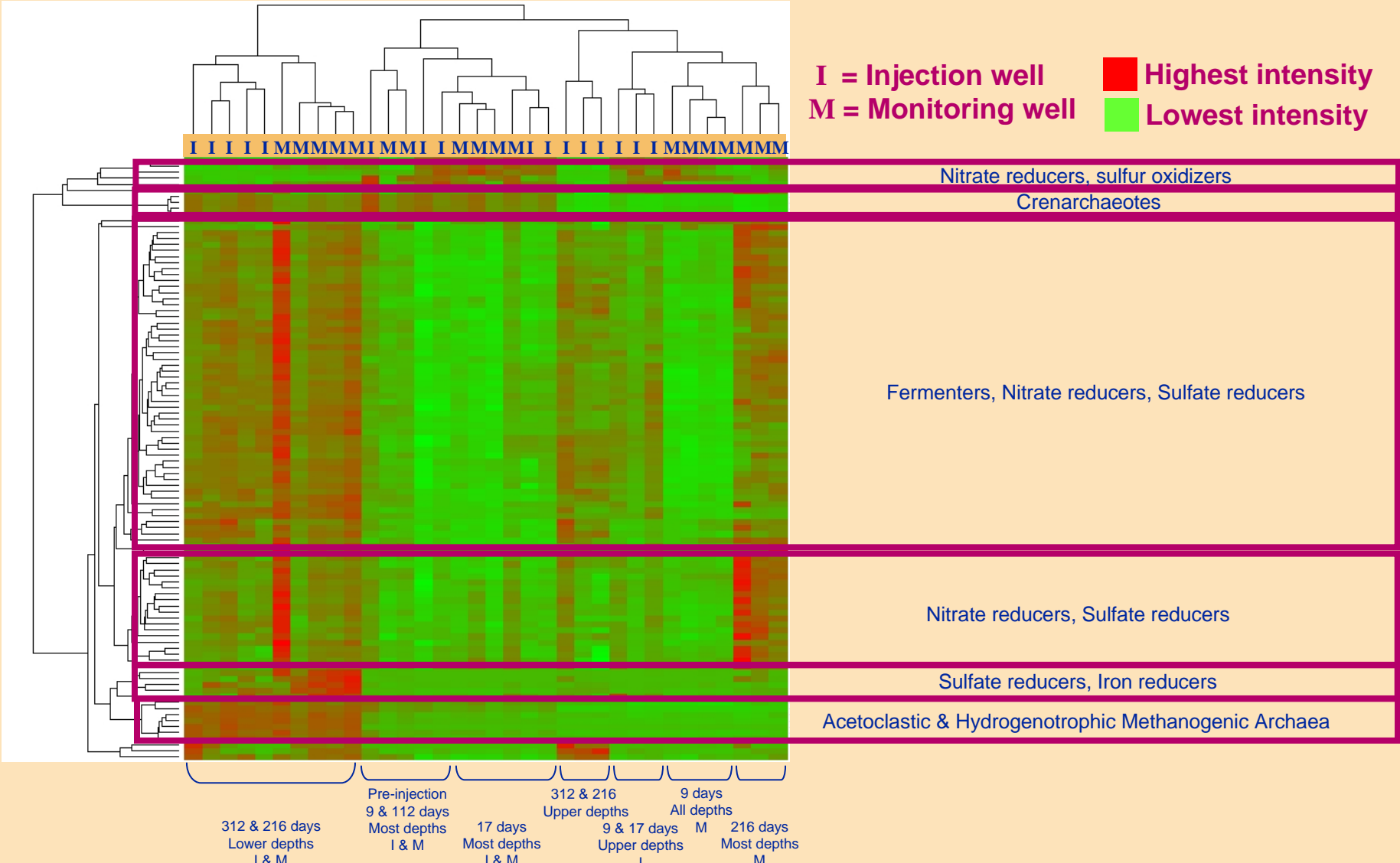


PhyloChip is scanned, fluorescence data analyzed and probe sets with >90% probes positive are considered present

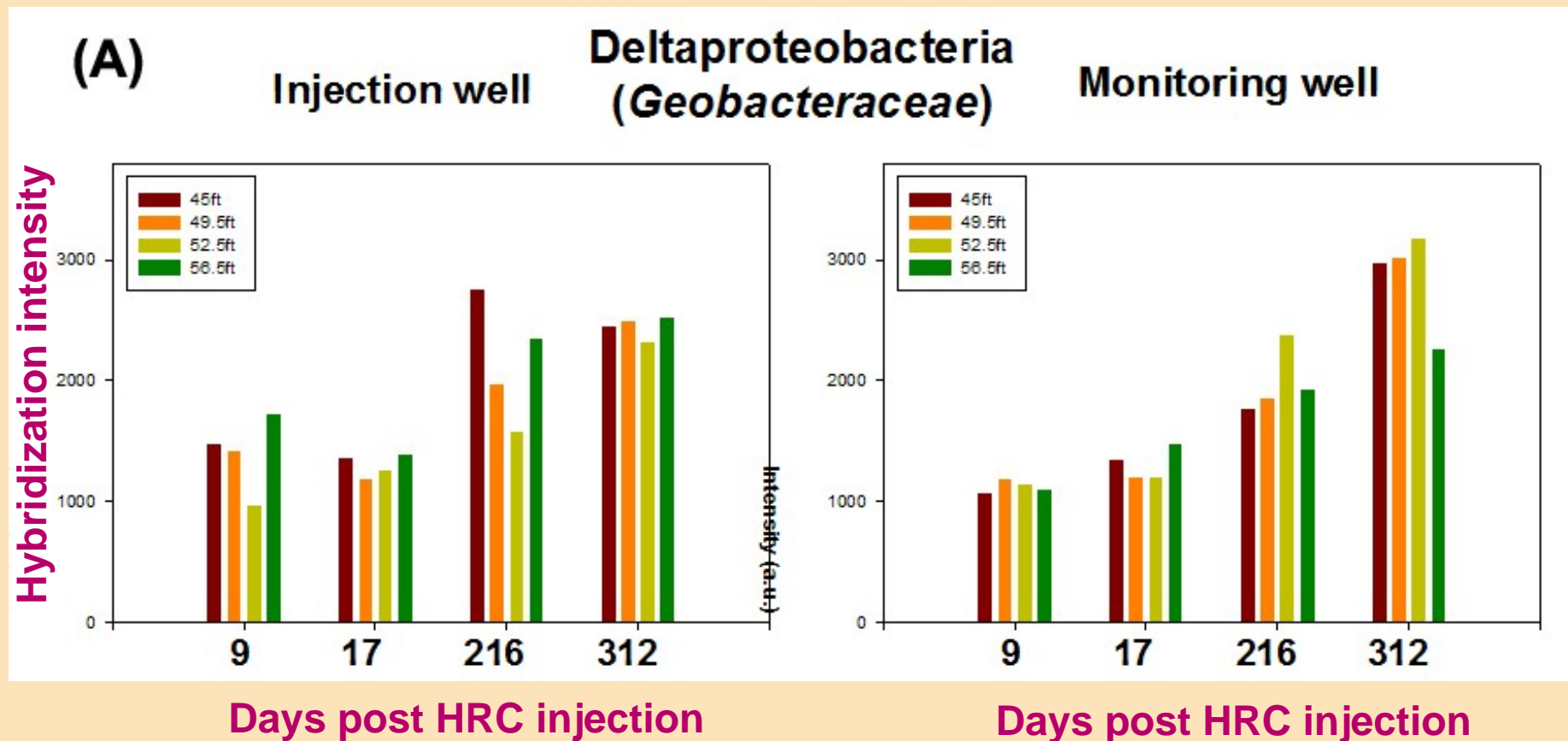
Microarray analysis of bacterial community changes during Cr(VI) remediation at Hanford 100H site:

Dynamics of some significant organisms.



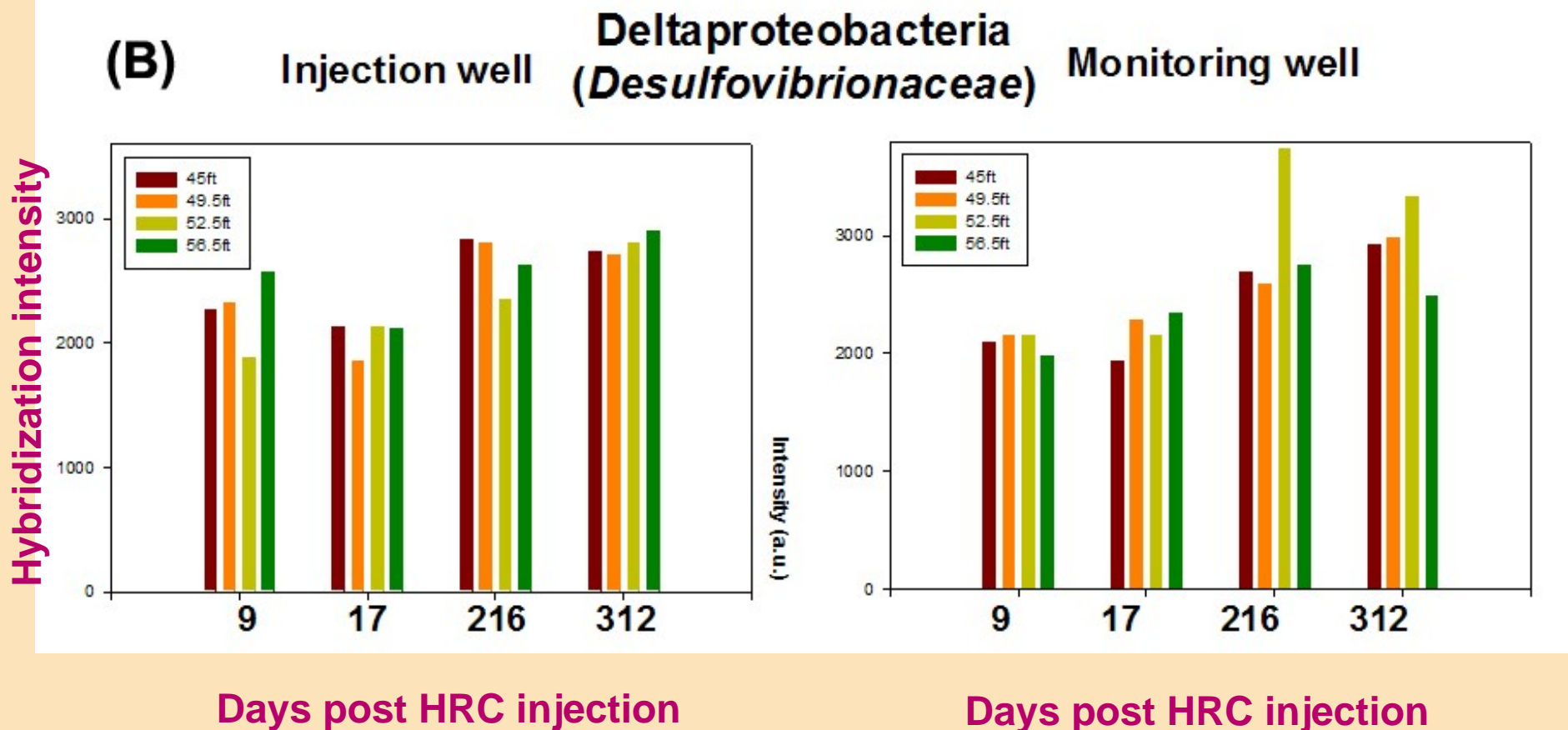


Functional groups - Iron reduction



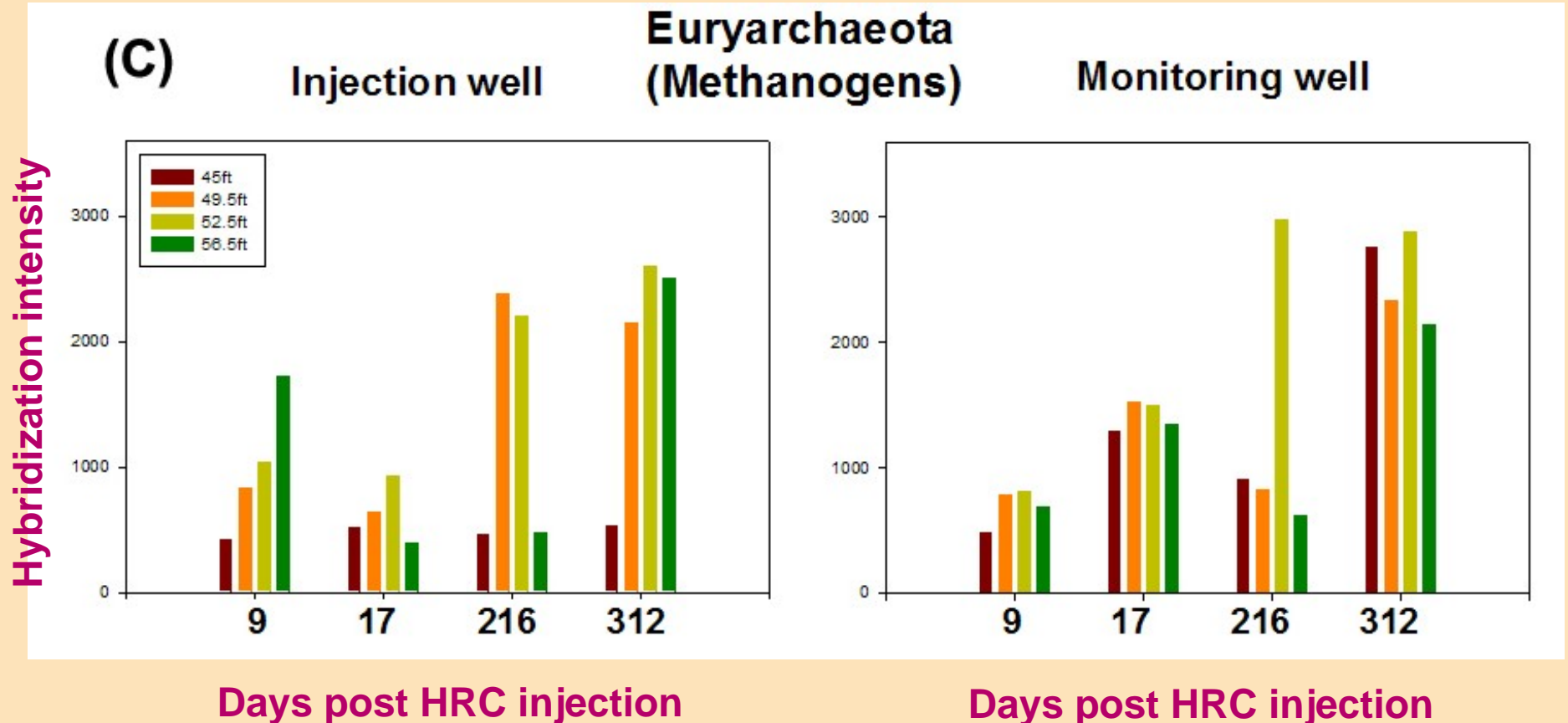
Fe(II) can abiotically reduce Cr(VI) to Cr(III)

Functional groups - Sulfate reduction



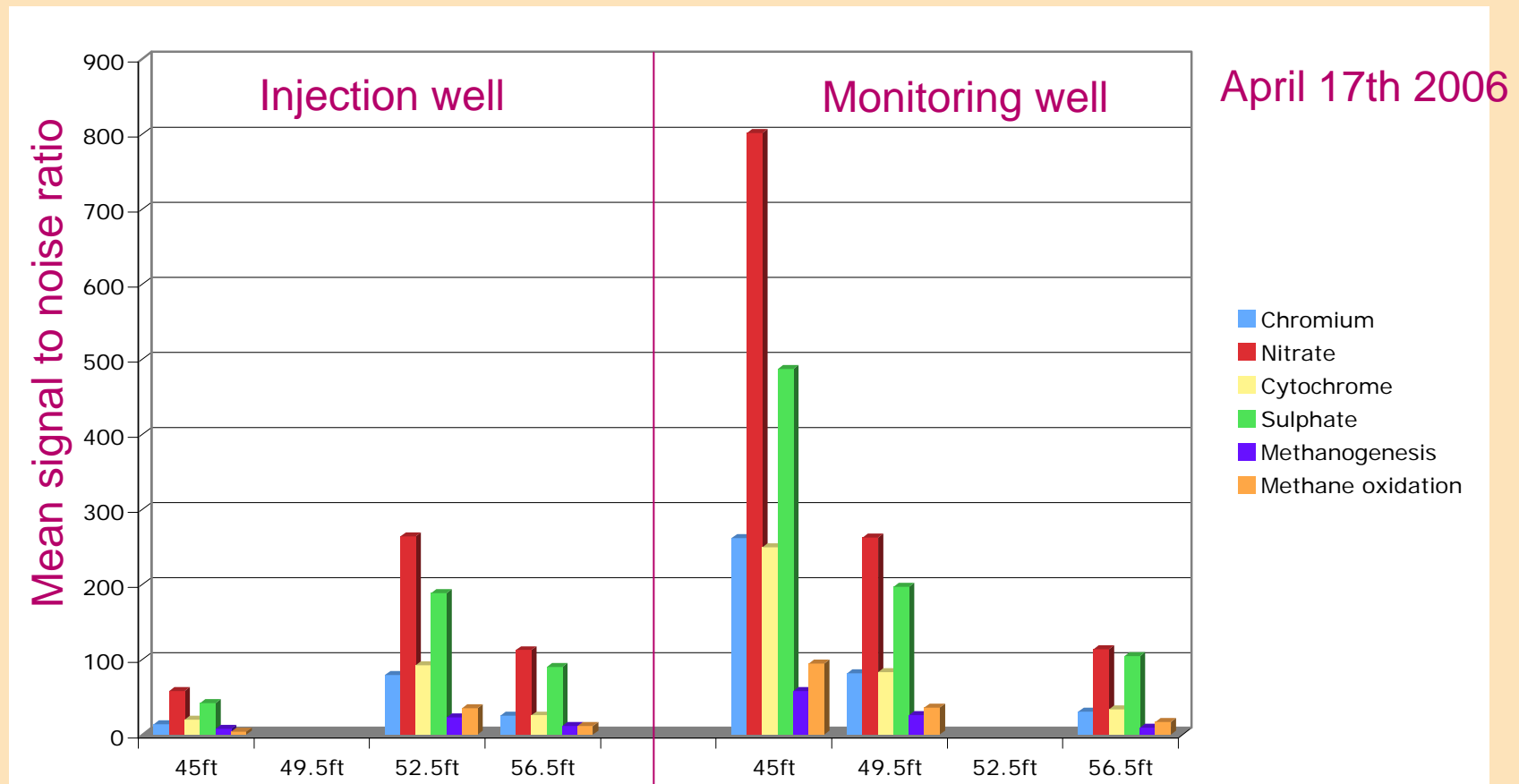
H_2S can abiotically reduce Cr(VI) to Cr(III)

Functional groups – Methanogenesis



Presence of methanogens indicates strongly reducing conditions

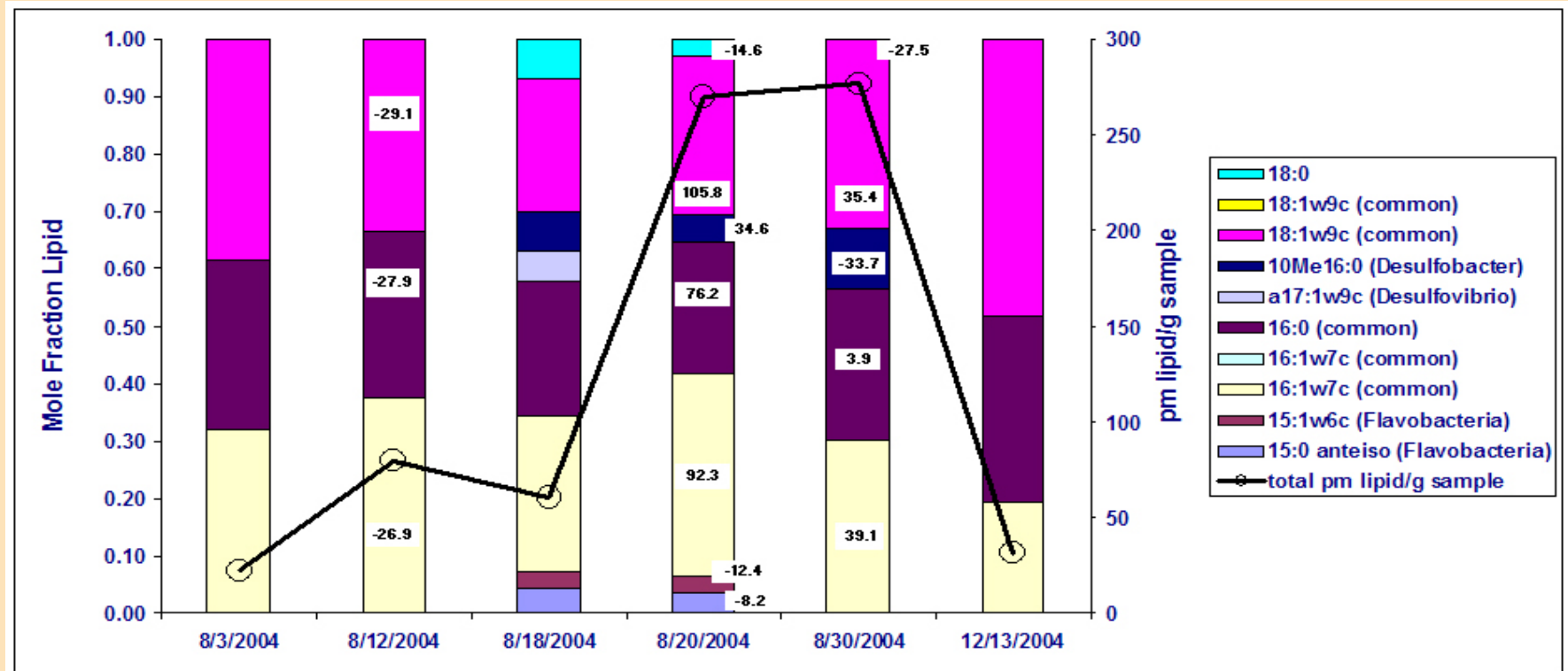
Functional microarray analysis



Nitrate, Sulfate, Iron reduction. Methanogenesis, Methane oxidation, Sulfur oxidation.
Many chromium tolerance/reduction genes.

Joe Zhou, Joy Van Nostrand - University of Oklahoma

^{13}C Phospholipid Analysis



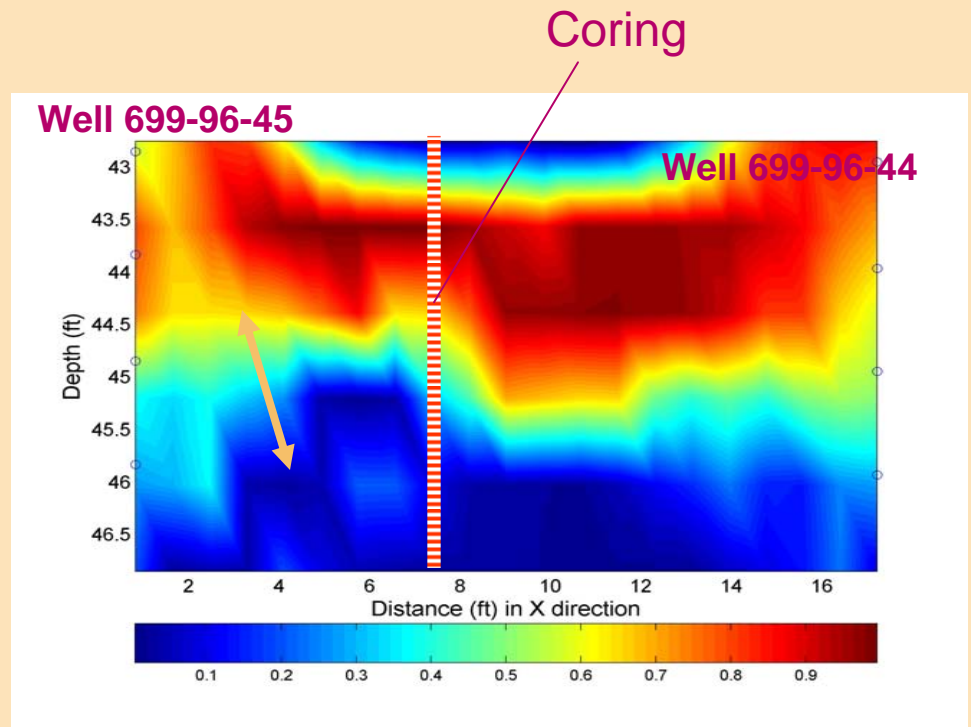
- General bacterial biomarkers indicate rapid enrichment in ^{13}C
- ^{13}C ratio is greater than expected (overall spiked HRC ratio was 15 per mil)
 - ^{13}C polylactate used as spike it is not esterified to glycerol backbone
 - it is released and consumed more rapidly
- Biomarkers for *Flavobacteriaceae* increased following injection but showed minimal enrichment with ^{13}C .
 - *Flavobacteria* do NOT typically utilize lactate, but may use glycerol (backbone, unlabeled)

Major Findings to Date

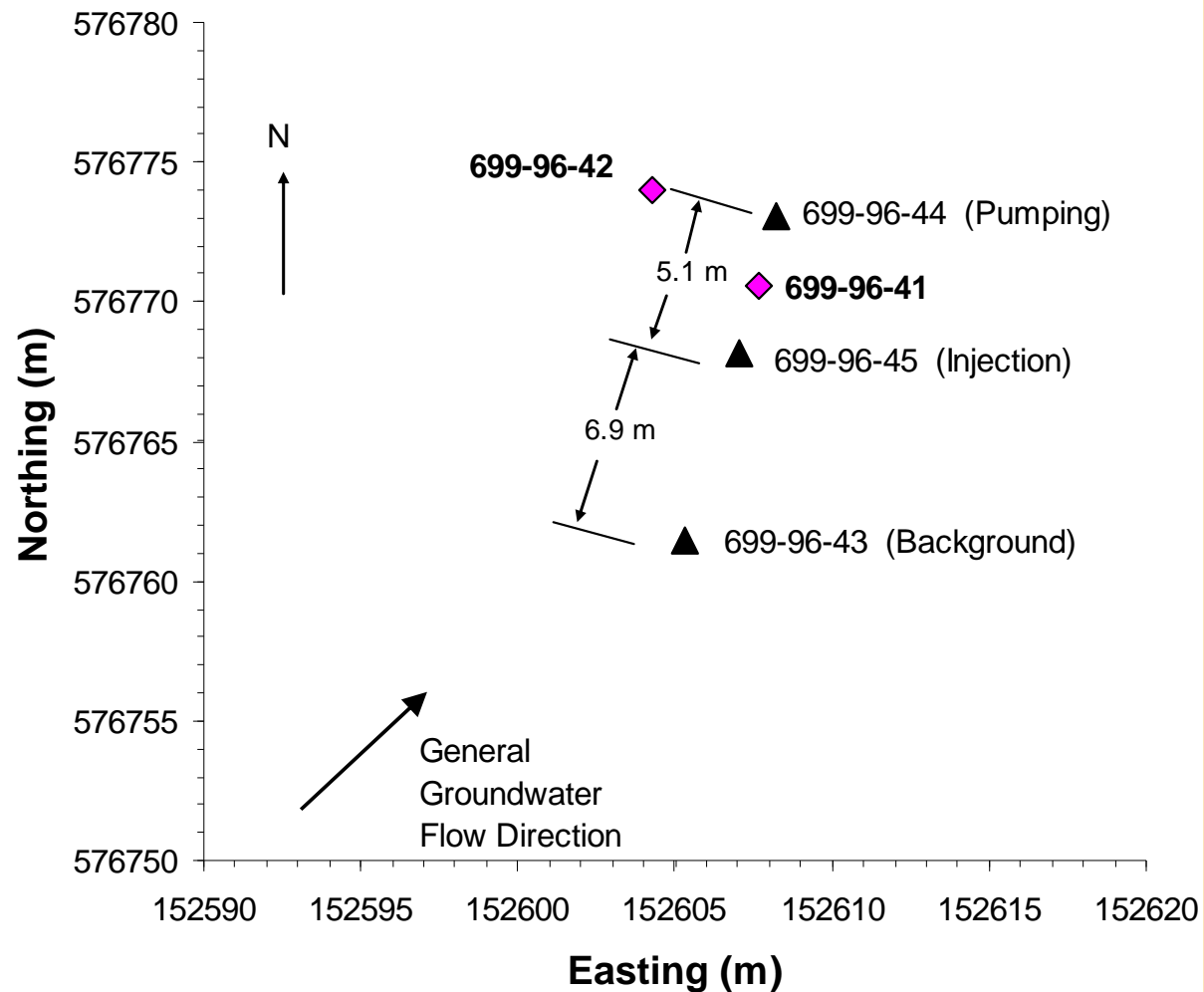
- Despite low initial microbial densities ($<10^5$ cells g^{-1}), HRC injection in the groundwater stimulated increase in the biomass up to $10^7 - 10^8$ cells ml^{-1}
- Highly reducing conditions were achieved quickly with hierarchical depletion of electron acceptors O , NO_3 , and $Fe(III)$ (SO_4 was reduced but never depleted except transiently months later), sulfate reduction has been sustained to for the last 20 months
- SIP analysis confirmed microbial metabolism of HRC and PLFA indicated which group of organisms was utilizing the electron donor
- Geophysical measurements were capable of characterizing hydrogeological conditions and monitoring the HRC distribution in groundwater
- Biostimulation has not yet had an effect on subsurface flow
- $Cr(VI)$ was reduced to drinking water standards after increases in $Fe(II)$, and has remained low for the last 20 months.

Future Research

- Metagenome Sequence by JGI
- Metagenome (large Insert and small insert clone libraries using MDA) by Diversa
- Isolation and sequencing of *Desulfovibrio* strains by JGI in the Lab Sequencing Program
- Mass transfer between high and low permeability zones
- Changes in hydraulic properties of sediments after HRC injection
- Evaluation of the potential for Cr(III) reoxidation
- Development of a numerical code TOUGH Bio-React
- Monitoring and new field tests (2 new wells installed over summer).



Layout of Wells at Cr Bioreduction Site



Contacts

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Hanford Project <http://esd.lbl.gov/ERT/hanford100h/>

ERSP <http://www.lbl.gov/ERSP>

Hazen Lab <http://www-esd.lbl.gov/ECO/Hazenlab/index.htm>

Ecology Department <http://www-esd.lbl.gov/ECO>

Center for Environmental Biotechnology <http://www-esd.lbl.gov/CEB>

Virtual Institute for Microbial Stress and Survival <http://vimss.lbl.gov>